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Characterisation of non-compendial impurity reference standards: How good is good enough?

QUALITY | ISO 9001 | ISO/IEC 17025
ISO 17034 | GMP

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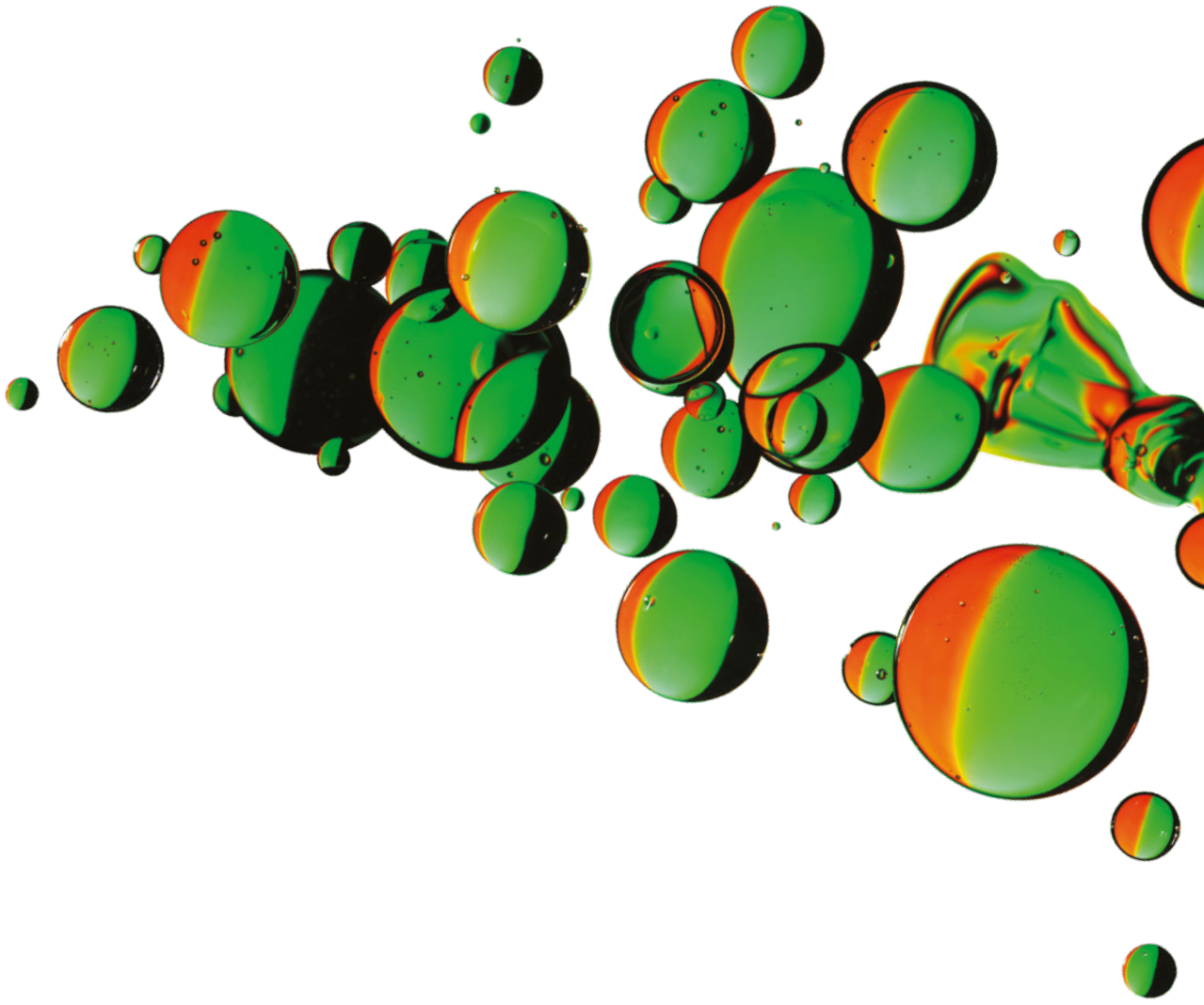
Abstract

Identification and quantification of impurities in pharmaceuticals are important stages in pharmaceutical development. Impurity reference standards (IRSs) play a vital role in these stages. This paper explains the different approaches to non-compendial IRSs, with a focus on analytical characterisation. The paper will also refer to risks connected to the quantitative use of an IRS which is designed for qualitative use only, or to the use of research materials for which the assay value is grossly overestimated. Such materials seem to be pervasively present in the market, as highlighted in a recent drug repurposing study by the US-based Broad Institute: 29% of an examined 8,500+ compound samples were of a purity below 85%, even when advertised as having much higher purity values. The risk of working with incorrect purity values can be mitigated by procuring IRSs from a recognised reference standards manufacturer.



Phase separation during the liquid-liquid extraction of Enalapril Maleate.
(Impurity Imidazole, MM0015.02)

1. Introduction

Identification and quantification of impurities in pharmaceuticals are important stages in the development of active pharmaceutical ingredients (APIs) and finished dosage forms (FDFs)[1]. Impurities must be controlled to levels that will ensure the quality of the product. The International Council on Harmonisation (ICH) Q3A and Q3B guidelines[2] for drug substances and drug products, respectively, provide regulatory expectations for investigation and control of impurities, including process-related substances and degradation products. Thresholds for identification and safety qualification of impurities can be based on relative percentages or directly in milligrams of exposure, depending on the nature of API and FDF. Table 1 comes directly from Q3A and provides an overview on thresholds for reporting, identification and qualification of impurities in APIs.

Qualification, according to Q3A/Q3B, is "the process of acquiring and evaluating data that establishes the biological safety of an individual impurity or a given impurity profile at the level(s) specified". In the event of exceeding the qualification threshold, the best case scenario would be that safety data is available from the literature; in the worst case, the data would have to be acquired by time-intensive and expensive toxicity studies. ICH rules also have to be followed for generic products. Guidance on this topic has been issued by the U.S. Food and Drug Administration (FDA)[3] and European regulatory authorities[4]. Monographs from the European Pharmacopoeia (Ph.Eur.) and United States Pharmacopoeia (USP) have been made compliant or are being made compliant to the ICH requirements, respectively. Therefore, an accurate assessment of impurity levels is needed during the research and development (R&D) phase of an API and/or FDF project.

Accurate impurity identification and quantification is also needed later in the lifecycle of the pharmaceutical product, i.e. during routine release testing.

During the R&D phase of new (generic) API and FDF projects, HPLC methods with MS and UV detectors are widely used for detecting, identifying and quantifying impurities. For routine quality control, HPLC methods with UV detection are developed and validated. In all these analytical steps, IRSs play a major role. But how should these reference standards be characterised?

For primary reference standards for APIs, numerous suggestions are available, such as in the general text 5.12. of the Ph.Eur, or ICH Guideline Q7[5]. However, for IRSs, information and guidance is less easily at hand, and less detailed. The Ph.Eur. states in text 5.12. that "reference standards are established using suitable procedures and their continued suitability for use is monitored", and further "a CRS (Chemical Reference Substance, author's note) corresponding to an impurity is characterised for identity and purity"[6]. The ICH simply requires in impurity guidelines Q3A/Q3B that "reference standards used in the analytical procedures for control of impurities should be evaluated and characterised according to their intended uses". National regulation authorities also do not provide further guidance on the topic. The German Federal Institute for Drugs and Medical Devices (BfArM) did issue guidance in their 1996 explanations about drug filing[7], noting in appendix 6 that: "Impurity standards are used for purity tests and during method development and validation of those tests. Identity must be ensured and purity and assay must be defined." However, this guidance is no longer valid, due to the transfer to European regulation.

Table 1.
Thresholds of ICH Q3A for reporting, identification and qualification.

Maximum Daily Dose	Reporting Threshold	Identification Threshold	Qualification Threshold
≤2g/day	0.05%	0.10% or 1.0mg per day intake (whichever is lower)	0.15% or 1.0mg per day (whichever is lower)
>2g/day	0.03%	0.05%	0.05%

[1] An FDF is often termed a drug product as well.
[2] ICH Q3A(R2), Q3B(R2).
[3] FDA Guidances for Industry, ANDAs: Impurities in Drug Substances/Products, https://www.fda.gov/downloads/Drugs/Guidances/UCM172002.pdf, https://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm072861.pdf, accessed March 13, 2018.
[4] EMA Guideline CPMP/QWP/1529/04, https://www.ema.europa.eu/documents/scientific-guideline/guideline-control-impurities-pharmacopoeial-substances-compliance-european-pharmacopoeia-general_en.pdf, accessed March 13, 2018.
[5] ICH Q7, glossary.
[6] Ph.Eur. General Text 5.12. Note: Assuring "continued suitability for use" as mentioned in Ph.Eur. General Text 5.12. requires an ongoing stability or "fit for purpose" monitoring programme – an important characteristic which distinguishes reference standards from research chemicals. Such programmes run under the quality accreditation of the RS manufacturer, ideally under ISO 17034:2016 (General requirements for the competence of reference material producers), representing the highest quality level possible.
[7] Erläuterungen zum Antrag auf Zulassung eines Arzneimittels beim BfArM, 1996, Appendix 6.

A clear and common requirement for IRSs is therefore lacking, and it is not surprising that approaches to impurity standards are highly variable, from both manufacturers of such standards and end users alike.

This paper looks at the different approaches to non-compendial IRSs[8], with a focus on analytical characterisation, and on what level of characterisation is adequate for its corresponding purpose. The paper will also highlight the risks connected to the quantitative use of an IRS which is characterised in a way that is designed for qualitative use only. The same dangers exist when using research materials with grossly overestimated assay values. The risk of encountering research materials with incorrect assay values can be alleviated by sourcing comprehensively documented impurity reference standards from a certified reference standards manufacturer[9].

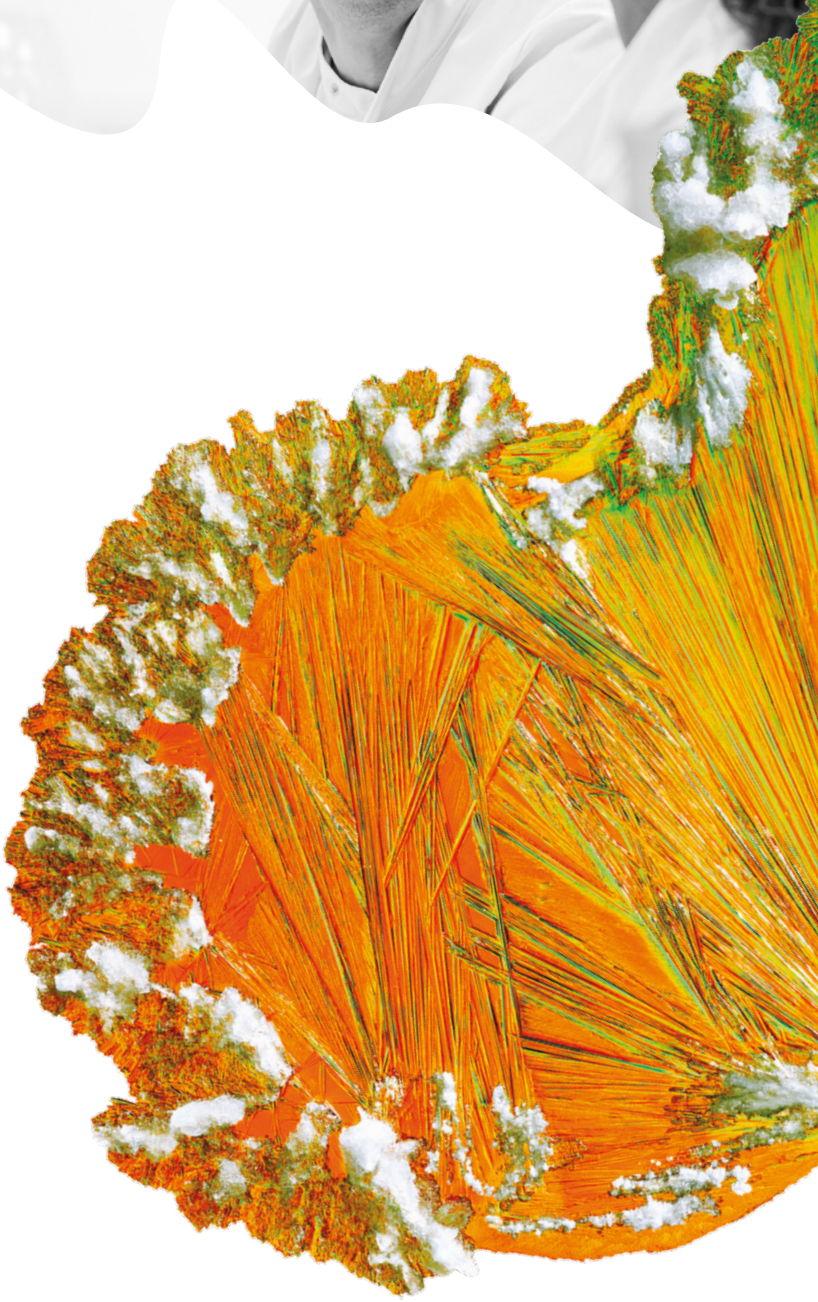
2. The intended analytical purpose

The major factor which should determine the extent of analytical characterisation of an IRS is the intended analytical purpose. There are two main types of analytical use: qualitative and quantitative.

2.1. Possible qualitative uses are

System suitability test, e.g. resolution check

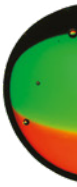
– These tests are often used in pharmacopoeial methods, but they also play an important role for in-house analytical procedures. Two or more analytes, often the API and one or more impurities that are eluting somewhat close together in the routine HPLC method, need to be separated in a sufficient way in order to assess the correct performance of the method in its current setting. So, prior to running the real samples and obtaining valid results, the whole system is checked for its suitability by running a mixture of these two (or more) analytes, expecting



Crystallization of Metformin Hydrochloride after removal of Methanol from the reaction mixture. (Impurity Cyanoguanidine, MM0056.01)

[8] Compendial impurity standards are out of scope here as they are intended for use only in combination with the procedures mentioned in the pharmacopoeial monographs/chapters. Their use for other purposes is not covered by the pharmacopoeias (e.g. USP general chapter 11, Ph.Eur. general text 5.12.).

[9] Interestingly, the US-based Broad Institute (www.broadinstitute.com) found in their drug repurposing project as a side result that 29% of the examined 8,500+ compound samples were of a purity below 85%, even for products that were announced with much higher values (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5568558/). It is a fair assumption that a similar rate of commercially available research chemicals show the same inconsistencies where purity is concerned. The risk of working with incorrect purity values can be mitigated by procuring IRSs from a recognised reference standards manufacturer, ideally operating under the ISO 17034:2016 quality system, as described in footnote 6.



certain minimum and pre-defined resolution criteria between adjacent peaks to be fulfilled. Having performed the critical separation successfully, the method is also assumed suitable to separate the other (specified) impurities sufficiently from each other.

Peak identification

– Unknown impurity peaks observed during process/method development are often looked at by mass spectrometry (MS) to obtain information on molecular mass and maybe also, by fragmentation patterns, chemical structure. These experiments deliver a tentative molecular structure, which can be confirmed by running a reference standard (whose identity has been unambiguously determined with more than just MS experiments) by the selected method, and demonstrating that the retention time, UV and MS spectra are the same as for the impurity peak in question.

Validation of specificity parameters

– ICH Q2 guideline[10] provides information on validation methodology, e.g. on how to validate the specificity of the API assay method. One suggestion of Q2 is to spike "pure substances (drug substance or drug product) with appropriate levels of impurities … demonstrating that the assay result is unaffected by the presence of these materials." This can be achieved by comparison of assay results on spiked versus unspiked samples, with the help of statistical means like F and t tests. Demonstrating separation of impurities from the API also supports method specificity.

2.2. Possible quantitative uses are:

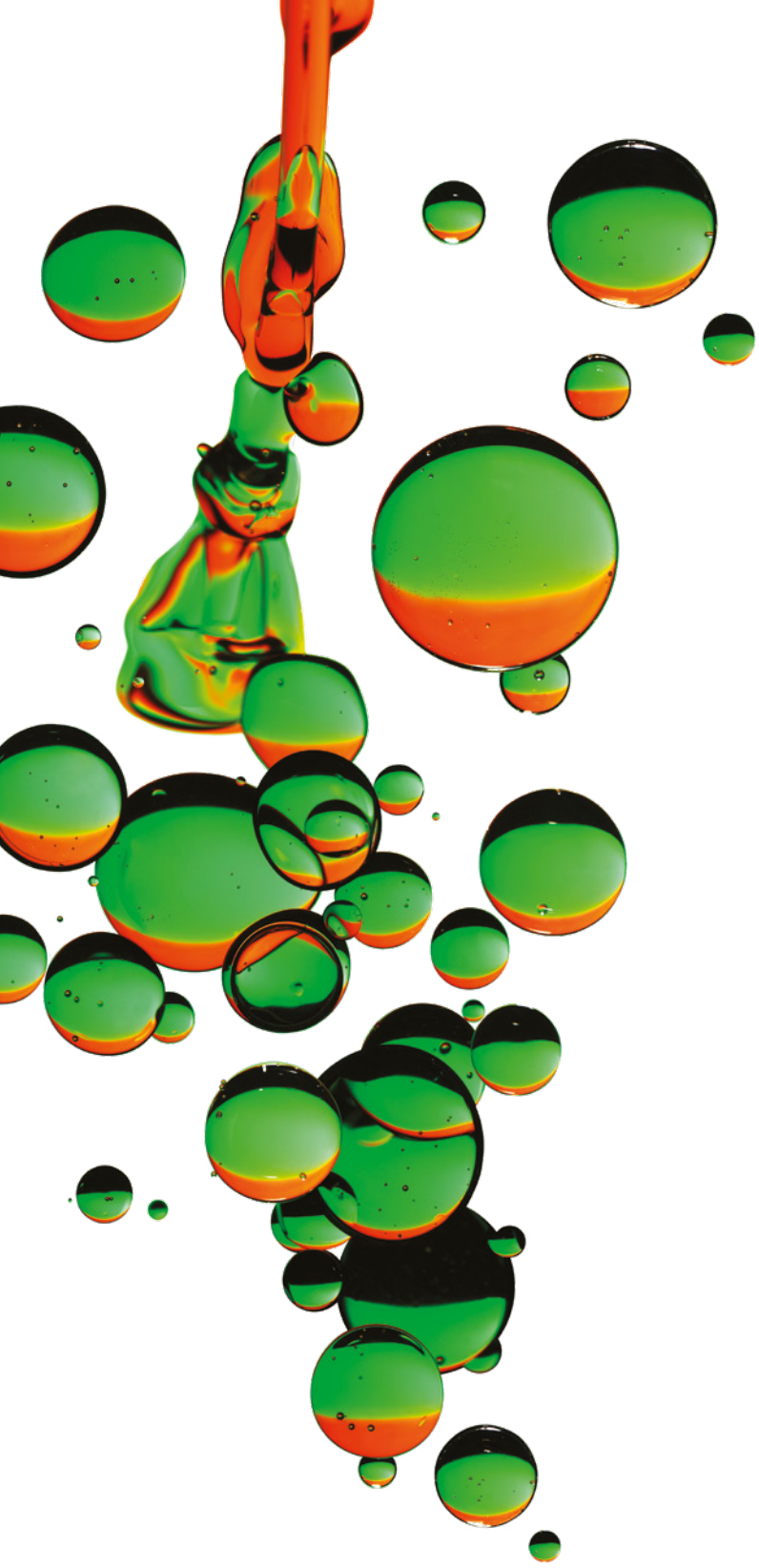
Limit test (semi-quantitative)

– Limit tests are very often included in pharmacopoeial monographs, and can also be developed for in-house impurity quantitation. In particular for unspecified and unidentified impurities it is typical to compare the "area under curve" of the impurity peaks with that of an appropriately diluted API test solution. Limit tests are also performed for specified impurities, often against a solution of the impurity under investigation. In Q2, limit tests require less validation effort compared to quantitative impurity determination. They can therefore be considered to be of semi-quantitative nature.

Quantification of impurity with the direct use of the IRS

– This is the typical use of an IRS in routine quality control. It is mainly applied for specified impurities, and then very often in those cases where the content of the impurity will increase over time (e.g. major degradation products). As for an API assay method, a calibration curve is recorded over the whole range of the method, using solutions of the IRS in varying concentrations. A single point calibration can only be used if the method is linear over the range of interest.

[10] ICH Q2(R1).



Quantification of impurity via relative response factors (RRFs)

– This topic is dealt with in detail in another white paper[11]. The development of RRFs is normally performed with the help of IRSs, thereby establishing a fixed correlation between the analytical response of an appropriately diluted solution of an API reference standard with the solution of an IRS. This correlation is expressed in the RRF value, which, when accurately assigned, can be used under certain circumstances to determine the impurity content. Potential ruggedness issues with RRFs should be considered when incorporating RRFs in methods for routine long-term use.

Validation of accuracy parameter

– ICH Q2 recommends that "accuracy should be assessed on samples (drug substance/drug product) spiked with known amounts of impurities." Accuracy is often evaluated using recovery experiments. The impurities are spiked in various amounts to cover the whole range of interest. The ICH recommendation is to validate over the range "from the reporting level of an impurity to 120% of the specification." Ideally the spiking should be performed with an IRS.

The next chapter will deal with the IRS characterisation necessary for the intended uses. Keep in mind that an IRS suitable for quantitative purposes is also applicable for qualitative uses, so if unsure in which direction the use of a certain IRS will develop, users should consider using quantitative material right from the start, thus securing consistency in results.

[11] See Mikromol white paper "External Reference Standards or Relative Response Factors: Considerations for Quantitation of Impurities in Pharmaceuticals"; https://www2.lgcgroup.com/MikromolWhitePapers.

3. Characterisation of qualitative and quantitative IRSs

3.1 Minimum requirements for qualitative use

The major point here is, of course, to make sure that the identity of the IRS candidate material is exactly what it should be, with regard to the organic moiety. In most cases it will not be necessary to also have information on the salt form (i.e. salt counterions) available. The analyte solvent and the mobile phase normally determine in which form the organic moiety passes through the HPLC column and is seen by the detector, so retention time, UV and mass spectra should be comparable between the IRS and sample peak, regardless of the salt counterions (if any) present in the IRS. Possible pH effects of salt forms and related shifts in retention times are normally suppressed by the use of robust buffer systems for the mobile phase.

It is important to confirm the identity of the IRS with not just one technique (compare also Ph.Eur. General Text 5.12.), but with a sensible combination of methods. In most cases, nuclear magnetic resonance (in particular 1H-NMR), infrared spectroscopy (IR) and MS data, the latter normally from HPLC- or GC-experiments, should give sufficient information on the chemical structure of the IRS candidate material. If the impurity is specified as an enantiomer, then its enantiomeric purity should be determined if it is used for quantitative chiral purposes.

Moreover, elemental analysis (CHN) can provide further support of chemical composition and can also give indications on possible salt forms.

Knowledge of potential salt forms can sometimes be derived from information, if available, about how the IRS was synthesised or isolated.

During interpretation of the identity data, no signal or result should conflict with the assumed chemical structure of the IRS. If contradictions arise, it is essential to resolve these, prior to a release of the candidate material. It is also recommended to aim for a minimum purity of 85%, otherwise interpretation of NMR and IR data can become difficult.

3.2 Minimum requirements for quantitative use

First the identity needs to be ensured, so everything stated above under 3.1. applies to quantitative standards as well. For quantitative applications, however, it is highly recommended to perform CHN analysis to check if the IRS candidate material is present as a salt form. Alternative methods can, of course, be used, but salt form verification is vital for the quantitative use of the IRS. Chromatographic purity might be high for both a free base/acid and a salt form of a candidate material, but there could be drastic differences in the assay value of the organic moiety. Several cases have been reported where this became an issue. There were even recalls of compendial reference standards in the recent past related to this topic.

Organic purity is the main requirement in the evaluation of an IRS. The purity should be as high as reasonably possible, as the 100%-method (i.e. mass balance) is based on determining all the impurity's impurities. The purer the compound, the lower the error of determining these impurities and the more accurate[12] the assay figure for the IRS. Organic purity is in almost all cases determined by chromatographic methods, mainly HPLC[13].

The organic purity does not equal the final assay value of an IRS. For example, the amount of water and residual solvents should be determined as well and subtracted from 100%.

Indications of inorganic impurities might have been obtained, and then further examined, by CHN. If not, residue on ignition (ROI)[14] can provide supplementary data, but it is seldom used in IRS characterisation because the technique consumes large amounts of valuable impurity candidate material. If desired, techniques like ion chromatography can be used to quantify inorganic impurities.

In the absence of inorganic impurities, the final assay can be calculated using equation 1:

Equation 1.

$$\text{Assay (\%)} = (100\% - \text{volatile contents}) \times \frac{\text{Purity(\%)}}{100\%}$$

For volatile contents both water and residual solvents are included. They are considered absolute contributions, whereas purity is considered a relative contribution.

[12] Accuracy is sometimes also called trueness (see also Q2(R1)).

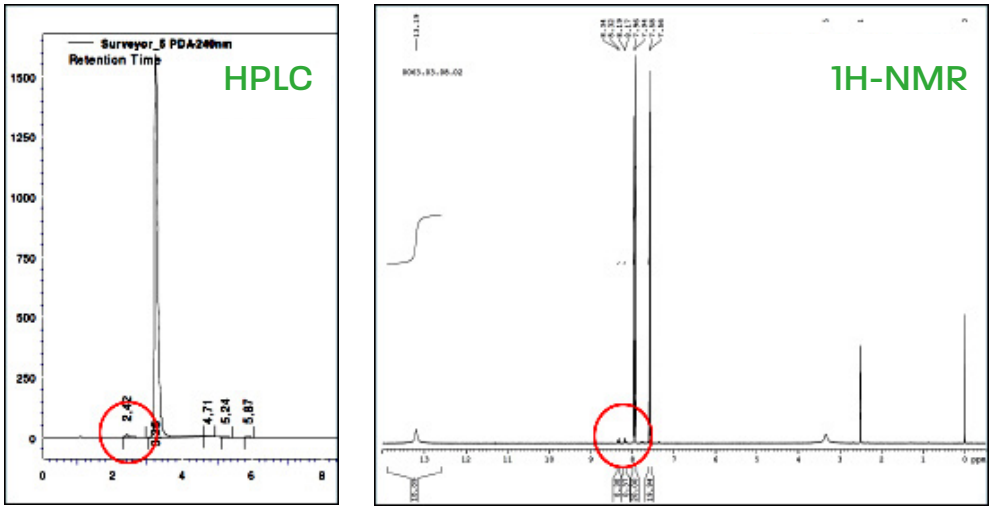
[13] That is also why organic and chromatographic purity are often used as synonyms.

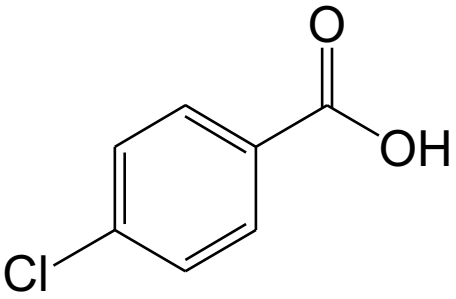
[14] In Europe ROI is better known as Sulphated Ash.

3.3 Consistency checks

During IRS production, there is often the possibility to confirm certain data by information derived via other examinations. For example, Figure 1 shows the HPLC chromatogram and 1H-NMR of an IRS for 4-chlorobenzoic acid, a bezafibrate impurity. Traces of the hydroxyl derivative can be observed in the HPLC at very low percentages. The NMR proves the presence of the hydroxyl derivative in exactly these trace quantities, confirming that the HPLC method conditions reflect purity correctly. In general it can be said that, if appropriate experience is present at the IRS manufacturing site, looking for consistency between orthogonal methods provides a powerful tool to ensure correct identity and assay.

Figure 1.
HPLC chromatogram and ^{1}H-NMR of an IRS for bezafibrate impurity A (EP).





4-Chlorobenzoic acid (MM0063.03),
bezafibrate impurity A (EP),
with traces of hydroxyl derivate

4. Use of qualitative IRSs for quantitative purposes

Unlike for API reference standards, it is possible to use qualitative IRSs for some quantitative uses, subject to the caveats below[15]. This is due to the fact that – under normal circumstances – a qualitative IRS taken as 100% pure will overestimate impurities in APIs and FDFs. ICH guidelines allow procedures that result in overestimating impurities, as there is no safety or quality concern for patients who are using the medicine. There may, however, be economic and validation concerns for users of the qualitative materials.

The user of a qualitative IRS should however consider the following caveats:

Qualitative IRSs should always be considered to be 100% pure.

– It is not a wise approach to use an analytical statement like "purity >80%" as assay value for calculation purposes[16]. The actual assay is normally higher, and underestimating the impurity in sample analysis cannot be tolerated in any GMP environment.

Issues during validation studies are possible.

– The use of an insufficiently characterised IRS during accuracy validation of the method for impurity testing (by recovery rates) can result in validating outside the considered range, without noticing[17].

Overestimation (OE) is an economic risk.

– OE can trigger false-positive out of specification (OOS) results, leading to unnecessary, time-intensive investigations. When a batch change for the IRS is due, the risk of OOS results is increased. Examination of OOS events is considered expensive: for simple investigations, financial figures of at least 3,000 USD are reported. Costs can easily rise to tens of thousands of USD per day, depending on the FTEs working on the issue. Ultimately, if the OOS issue is not resolved appropriately, a whole production batch could be lost, adding further to the expense[18].

  During the R&D phase of new API/FDF projects, OE may lead to exceeding the qualification thresholds of ICH Q3A/B, triggering lengthy and costly toxicity studies. Q3A/B mention study durations of up to 90 days; adding another month for data evaluation after the study period means that these studies can in some cases delay the time to market by four months.

  The costs incurred by OOS investigations and toxicity studies are undoubtedly several times higher than the costs of investing in a quantitative IRS.

  It should be noted that the fewer analytical details available, the higher the economic risk. Depending on the source, qualitative standards often lack correct identity with regard to the salt form. Water and residual solvents are also often not checked for. Water in hydrates in particular can contribute a considerable percentage of the candidate material at hand. Salt form and water issues can easily lead to overestimation errors of 40% and more, i.e. assuming 100% assay when it is in fact 70% 'as is', even if the chromatographic purity is quite high.

[15] The qualitative IRS should not be confused with a research chemical (see also footnotes 6 and 11). Technically, a research material could be used, but a qualitative IRS set up under a dedicated quality system has normally undergone a higher level of identity checks, and is also monitored for its continuous fitness for use (i.e. stability testing).

[16] A survey held during an LGC webinar showed that only 16% of the replying participants (7 out of 44) understood the idea of calculating with 100%.

[17] Example: A reference standard has a 70% assay instead of 100% and is used for recovery rate purposes (spiking experiments). Then – for an impurity specification of NMT 0.15% – the validation would not take place in the recommended range of 0.05-0.18%, but from 0.04-0.13% only. The value of 0.13% would be below the specification of NMT 0.15%.

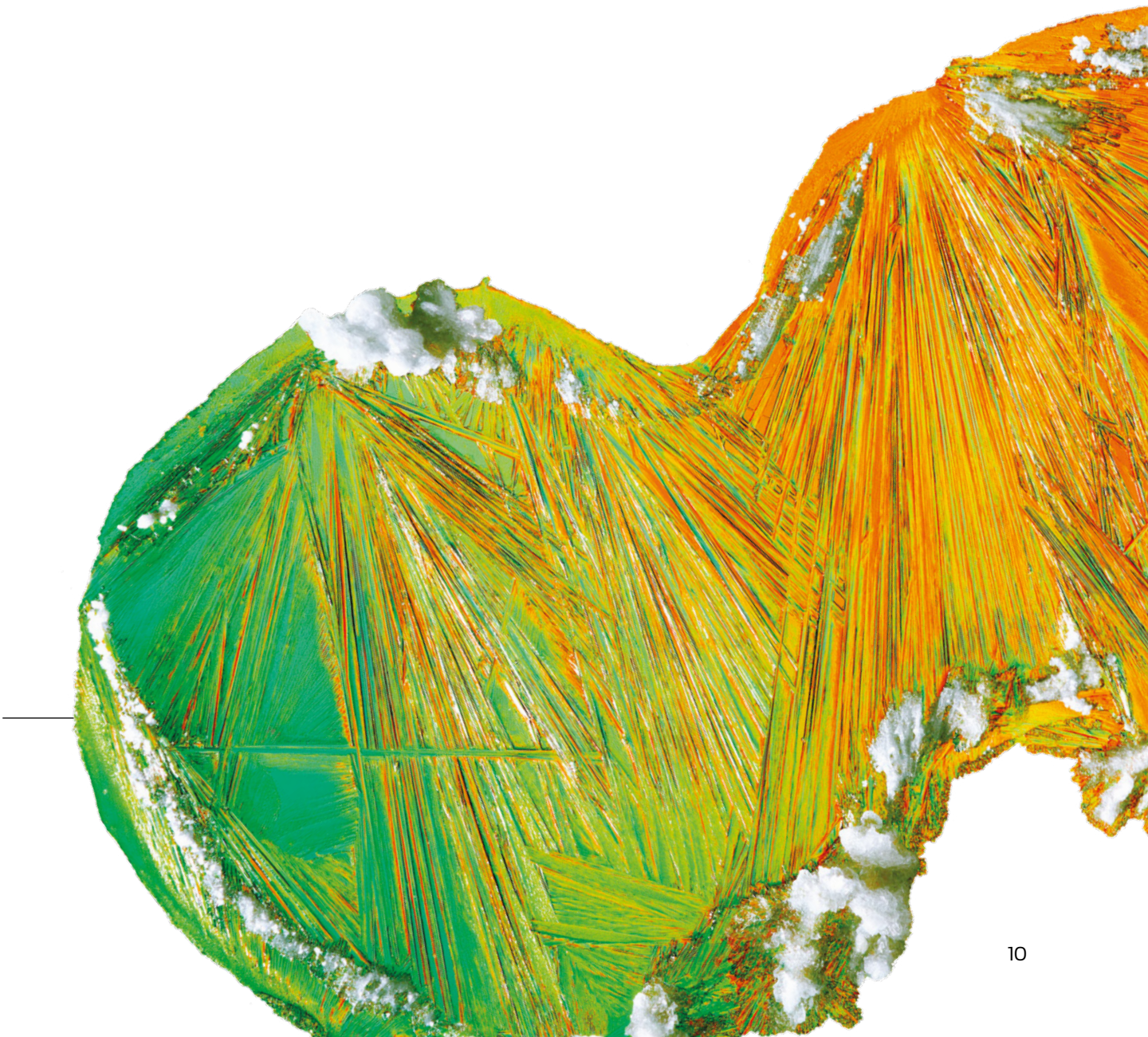
[18] How painful are OOS investigations?, http://complectors.com/?p=64, accessed March 9, 2018.

Conclusions

Identification and quantification of impurities in pharmaceuticals are important steps in the lifecycle of APIs and FDFs. IRSs are an important tool in identification/quantification of impurities.

For characterisation of IRSs, the intended purpose determines the minimum analytical effort necessary to provide a suitable reference standard. Qualitative IRSs need less analytical examination than those used for quantitative purposes. For the latter, you need an accurate assay determination, so it is essential to know if the salt form or free base/acid of a certain organic moiety is present as the candidate material. Furthermore, in addition to organic purity, water and residual solvents should be determined, to accurately calculate the assay via the mass balance approach.

Qualitative IRSs can be used – under certain conditions – for quantitative purposes as well. But the approach does lead in general to overestimation of impurities in APIs and FDFs, which is likely to cause additional costs that will be several magnitudes higher than the expense of a quantitative IRS. However, a well characterised quantitative reference standard, ideally produced under a dedicated quality system (i.e. ISO 17034:2016), is fit for all applications.



Oxidation of Ufiprazole with Hydrogen Peroxide during the synthesis of Omeprazole. (Impurity 5-Methoxy-2-[[(4-methoxy-3,5-dimethylpyridin-2-yl)methyl]sulphonyl]-1H-benzimidazole (Omeprazole Sulphone), MM0095.05)

About the authors

Bernard A. Olsen, Ph.D., received his undergraduate degree from Nebraska Wesleyan University in chemistry and his doctorate in analytical chemistry from the University of Wisconsin-Madison. He is an independent consultant providing expertise in chemistry, manufacturing, and control to the pharmaceutical industry. He has over 37 years of experience in drug development including 29 years at Eli Lilly, where he was a Senior Research Fellow and contributed to the development and support of over 25 commercial drugs and numerous developmental drugs. He has published and given invited lectures on a wide array of drug development and analytical topics including high performance liquid chromatography, method development and validation, impurity determination and control, genotoxic impurities, physical property characterisation, drug counterfeiting, regulatory aspects of drug development, and quality control. He is also a Fellow of the American Association of Pharmaceutical Scientists.

Dr. Christian Zeine studied chemistry at the Westphalian Wilhelms-University in Münster, Germany, where he received his doctorate in 1998 in organosilicon chemistry. After project work at the University, from 1999 on there followed activities at manufacturers of medical and in-vitro diagnostics products, including B. Braun Melsungen. Since 2002 he has worked at LGC Standards, serving as Global Senior Product Manager for pharmaceutical reference standards.

Christian is also the author of articles introducing the general topics of impurity testing and reference standards, and lectures on these subjects during seminars and symposia.

About Mikromol
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At Mikromol we combine an incomparable depth of pharmaceutical knowledge and 25 years of manufacturing experience to ensure our portfolio of over 5,000 Impurity, Active Pharmaceutical Ingredient (API) and Excipient reference standards is of the highest quality. We go beyond the standard, supporting you with the highest accreditation for reference standard purity and a comprehensive Certificate of Analysis, to help you achieve greater analytical certainty.

Mikromol: making a positive, measurable difference.

About LGC
lgcgroup.com

LGC is an international leader in the extended life sciences sector, providing a comprehensive range of reference materials, proficiency testing schemes, genomics reagents and instrumentation, as well as research and measurement services. We have cutting-edge expertise in measurement science, serving as the UK National Measurement Laboratory and Designated Institute for chemical and bio measurement. Operating out of 19 countries worldwide, our reference material manufacturing capability includes five facilities accredited to ISO 17034 or its predecessor ISO Guide 34, ensuring our products remain best in class.

Mikromol™

Acetylation of 2,6-Dimethylaniline during the synthesis of Lidocaine.
(Impurity N-(2,6-Dimethylphenyl) acetamide, MM0102.08)